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FINAL REPORT

B-VITAMIN CONTENT OF INNER BARK TISSUE OF LOBLOLLY PINE: EFFECT OF INFESTATION BY THE SOUTHERN PINE BEETLE AND ITS SYMBIOTIC MICROORGANISM COMPLEX

Prepared by:

J Robert Bridges 12/17/81
J. R. Bridges — Date
Research Entomologist

Approved by:

Peter L. Lorio, Jr. 12/18/81
Peter L. Lorio, Jr. — Date
Project Leader
RWU 2203

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INTRODUCTION

The microbial associates of the southern pine beetle, *Dendroctonus frontalis* Zimm. (SPB), include 2 species of fungi that are transported and propagated in a specialized structure (mycangium) of the female beetle (Barras and Perry 1972). One fungus is an unidentified Basidiomycete; the other is *Ceratocystis minor* var. *barrasii* (Barras and Taylor 1973). Many other microorganisms including yeasts and bacteria are also found with SPB.

The mycangial symbionts are necessary for normal growth and development of SPB (Barras 1973). The basis for this obligatory symbiosis is not known, but a nutritional role for the symbionts is possible. The beetle-microorganism complex increases the total nitrogen concentration of loblolly pine inner bark (Hodges et al. 1968). Growth of the beetle-microorganism complex also results in a decrease in the reducing sugar content of inner bark tissue (Barras and Hodges 1969). These results suggest that the SPB-microorganism complex may benefit the beetle by increasing the normally low level of nitrogen in the inner bark or by decreasing the C : N ratio.

Because all immature insects probably require some of the B-vitamins (House 1965), these nutrients could be important in SPB development. Microorganisms, especially yeasts, are known sources of the B-vitamins (Thompson 1942, Harrow and Mazur 1962) and could make a significant contribution to the B-vitamin content of SPB food.

This study was conducted to compare the B-vitamin content of healthy loblolly pine inner bark to bark that has been infested with SPB and its symbiotic microorganism complex to ascertain whether the symbiotic microorganisms provide the SPB with B-vitamins.

MATERIALS AND METHODS

Preliminary experiments were conducted to adapt standard procedures for B-vitamin analysis to the analysis of 5 B-vitamins in loblolly pine inner bark. Tests were conducted with inner bark infested with SPB/microorganism complex. Bark was collected within 1-5 mm of SPB larval galleries. Notes on the vitamin analyses are given in the Appendix.

CONCLUSIONS

This study could not be completed because of the difficulties encountered in assaying vitamins in bark samples. Standard methods available for vitamin analysis were developed for food materials or other biological materials containing relatively high concentrations of vitamins. These standard methods were not adequate for analyzing the low levels of B-vitamins in pine bark. The analysis of most of the vitamins was further complicated by colored substances and other compounds in the bark that interfered with the assays.

There are no published procedures for vitamin analysis in tree bark or related materials. To adapt available standard methods to analysis of bark would require a substantial investment in time and effort. Our preliminary tests indicated that the levels of vitamins in infested bark are very low. Thus, any differences between infested and uninfested bark would be very small. The results would be hard to evaluate, of questionable accuracy, and of questionable importance. When weighed against the possible knowledge gained from completing the study, the additional investment in time does not seem to be justified.

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APPENDIX

Notes on Vitamin Analyses

1. Thiamine (Assoc. Vitamin Chemists, p. 123-145)

Chemical Method (Thiochrome Method)--This method involves extracting the sample in hot HCl. After extraction the sample was treated with a phosphatase enzyme to convert bound thiamine to its free form. We tried two enzymes--Mylase P (ICN) and takadiastase (Park-Davis). Mylase P gave poor results even with standard samples of brewer's yeast. This enzyme decreased the amount of thiamine in samples. Takadiastase gave better results, but based on the analysis of brewer's yeast, it did not seem to release all of the bound thiamine.

Following enzyme treatment the sample was poured over a Decalso column which absorbs thiamine thereby separating it from substances which might interfere with thiamine detection. Bark extracts were cloudy and would not flow through the Decalso. To remove these interfering substances we tried two methods. One was absorption of interfering substances by Celite 545. Celite 545 did not clear the samples. The other method was precipitation. For precipitation, the pH of samples was adjusted above, below, and back to 4.5 several times until a precipitate formed. The precipitate was removed by centrifugation. Precipitation helped to clear the samples enough so they would flow over the Decalso column.

All attempts at detecting thiamine in bark samples were unsuccessful. The reasons are: a) thiamine, if present, in bark is below the detection limit of the assay; b) enzyme preparations were ineffective in releasing thiamine from its bound form; or, c) interfering compounds and their

attempted removal may have caused thiamine to be masked, lost or destroyed.

2. Niacin (Assoc. Vitamin Chemists, p. 186-196)

a. Chemical Method I--This method is based on the reaction of niacin with cyanogen bromide to produce a colored compound. Bark samples contain colored substances which interfered with the test. For this method extracted niacin was partially purified and removed from solution by absorbing it on Lloyd's reagent. This step was necessary to remove interfering compounds. In our samples niacin was not absorbed to the reagent, possibly because of other compounds in the bark. Carbon was also tried as a decolorizing agent, but it also absorbed niacin.

b. Chemical Method II--This procedure worked better than Method I. Colored compounds from the bark did not seem to interfere significantly with niacin detection. In two separate analyses, we found 5.9 and 11.2 ppm of niacin in SPB infested bark.

c. General Enzymatic Procedure (Assoc. Vitamin Chemists, p. 154)--This procedure is designed to extract several B-vitamins at one time so that all B-vitamins can be measured on the same sample. Using this extraction procedure and Chemical Method II for assaying niacin, we found 3.4 and 4.6 ppm in two samples of infested bark.

One problem with this procedure was that the enzyme used in the extraction contained about half as much niacin as the sample. This, of course, decreased the accuracy and precision of the assay. Niacin content of bark could probably have been measured. However, a relatively large sample would have been required, and none of the other B-vitamins

could have been measured on the same sample.

3. Riboflavin

a. Fluorometric Method (Assoc. Vitamin Chemists, p. 160-167)--The procedure involves reacting the vitamin to produce fluorescent compounds which are measured with a fluorometer. Bark samples contained colored compounds that interfered with the procedure. We were unable to rid the samples of these interfering substances.

b. Lumiflavin Method (Strohecher and Henning 1965)--This method was tested using a 10 g sample of infested bark. We detected 0.69 ug/g of riboflavin in the sample. One problem was that the enzyme preparation used for extraction also contained riboflavin.

The primary problem with riboflavin analysis is that the low levels apparently present in pine bark would necessitate very large samples for accurate analyses.

4. Pyridoxine (Gyorgy and Pearson 1967)

The procedure used was a microbiological assay using *Saccharomyces carlsbergensis*. Our preliminary tests indicated that this procedure worked well for analysis of pyridoxine in bark samples. We followed the given procedure without modification and in a 1 gm sample of infested bark we found 3.6 ppm of pyridoxine; uninfested bark had 3.2 ppm.

5. Pantothenic Acid (Gyorgy and Pearson 1967)

This procedure is a microbiological assay using *Saccharomyces carlsbergensis*. For this procedure, alkaline phosphatase enzyme was used to free pantothenic acid from its bound form. The enzyme preparation

contained significant amounts of pantothenic acid. Because bark samples apparently contained very low amounts of pantothenic, if any, the amount in the enzyme was enough to obscure any of the vitamin that might have been in the bark. We tried several methods as suggested by the procedure to clean up the enzyme preparation, but we were not successful.

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